

Kip3A

864 aa full length sequence

The motor domain is amino acids **5**–348 or base pair **143**–1174.

5

Fig. 1A

5	601	GGTGTATAATGAACAGATCCATGACCTCCTGGAGCCCAAGGGGCCCCTTGCCATCCGCGA -----+-----+-----+-----+-----+-----+	660
		uValTyrAsnGluGlnIleHisAspLeuLeuGluProLysGlyProLeuAlaIleArgGl	
10	661	GGACCCCGACAAGGGGGTGGTGGTGCAGGACTTTCTTTCCACCAGCCAGCCTCAGCCGA -----+-----+-----+-----+-----+-----+	720
		uAspProAspLysGlyValValValGlnGlyLeuSerPheHisGlnProAlaSerAlaGl	
15	721	GCAGCTGCTGGAGATACTGACCAGGGGAACCGTAACCGCACGCAGCACCCCACTGATGC -----+-----+-----+-----+-----+-----+	780
		uGlnLeuLeuGluIleLeuThrArgGlyAsnArgAsnArgThrGlnHisProThrAspAl	
20	781	CAACGCGACTTCCTCCCGCTCCCATGCCATCTTCCAGATCTTGTGAAGCAGCAGGACCG -----+-----+-----+-----+-----+-----+	840
		aAsnAlaThrSerSerArgSerHisAlaIlePheGlnIlePheValLysGlnGlnAspAr	
25	841	GGTTCCAGGACTGACCCAGGCTGTCCAGGTGGCCAAGATGAGCCTGATTGACCTGGCTGG -----+-----+-----+-----+-----+-----+	900
		gValProGlyLeuThrGlnAlaValGlnValAlaLysMetSerLeuIleAspLeuAlaGl	
30	901	CTCAGAGCGGGCATCCAGCACCCATGCGAAGGGGGAGCGGCTGCGGGAGGGGGCCAACAT -----+-----+-----+-----+-----+-----+	960
		ySerGluArgAlaSerSerThrHisAlaLysGlyGluArgLeuArgGluGlyAlaAsnIl	
35	961	CAACCGCTCTCTGCTGGCGCTCATCAACGTCCTCAATGCCTTGCCCGATGCAAAGGGCCG -----+-----+-----+-----+-----+-----+	1020
		eAsnArgSerLeuLeuAlaLeuIleAsnValLeuAsnAlaLeuAlaAspAlaLysGlyAr	
40	1021	CAAGACCCATGTGCCCTACCGGGACAGCAAAGTACCCGCCTGCTCAAAGACTCCCTCGG -----+-----+-----+-----+-----+-----+	1080
		gLysThrHisValProTyrArgAspSerLysLeuThrArgLeuLeuLysAspSerLeuGl	
45	1081	GGGCAACTGCCGCACAGTGATGATCGCTGCCATCAGCCCCTCCAGCCTGACCTACGAGGA -----+-----+-----+-----+-----+-----+	1140
		yGlyAsnCysArgThrValMetIleAlaAlaIleSerProSerSerLeuThrTyrGluAs	
50	1141	CACGTACAACACCCTCAAATATGCCGACCGGGCCaAGGAGATCAGGCTCTCGCTGAAGAG -----+-----+-----+-----+-----+-----+	1200
		pThrTyrAsnThrLeuLysTyrAlaAspArg Ala LysGluIleArgLeuSerLeuLysSe	
55	1201	CAATGTGACCAGCCTGGACTGTCACATCAGCCAGTATGCTACCATCTGCCAACAGCTCCA -----+-----+-----+-----+-----+-----+	1260
		rAsnValThrSerLeuAspCysHisIleSerGlnTyrAlaThrIleCysGlnGlnLeuGl	

Fig. 1B

5
1261 GGCTGAGGTAGCCGCTCTGAGGAAGAAGCTCCAAGTGTATGAGGGGGGAGGCCAGCCCCC
-----+-----+-----+-----+-----+-----+
nAlaGluValAlaAlaLeuArgLysLysLeuGlnValTyrGluGlyGlyGlyGlnProPr 1320

10
1321 ACCACAGGACCTCCCAGGATCTCCCAAGTCGGGACCACCACCAGAACACCTTCCCAGCTC
-----+-----+-----+-----+-----+-----+ 1380
oProGlnAspLeuProGlySerProLysSerGlyProProProGluHisLeuProSerSe

15
1381 CCCCTTGCCACCCACCCCTCCCAGCCAGCCCTGCACCCCAGAGCTCCCTGCAGGGCCTAG
-----+-----+-----+-----+-----+-----+ 1440
rProLeuProProHisProProSerGlnProCysThrProGluLeuProAlaGlyProAr

20
1441 AGCCCTTCAAGAGGAGAGTCTGGGGATGGAGGCCAGGTGGAGAGGGCCATGGAAGGGAA
-----+-----+-----+-----+-----+-----+ 1500
gAlaLeuGlnGluGluSerLeuGlyMetGluAlaGlnValGluArgAlaMetGluGlyAs

25
1501 CTCTTCAGACCAGGAGCAGTCCCCAGAGGATGAGGATGAAGGCCAGCTGAGGAGGTTC
-----+-----+-----+-----+-----+-----+ 1560
nSerSerAspGlnGluGlnSerProGluAspGluAspGluGlyProAlaGluGluValPr

30
1561 AATCCAGATGCCAGAGCAGAATCCACACATGCACTGCCAGAGTCCCTCGCCTGACCTT
-----+-----+-----+-----+-----+-----+ 1620
oThrGlnMetProGluGlnAsnProThrHisAlaLeuProGluSerProArgLeuThrLe

35
1621 GCAGCCCCAAGCCAGTCGTGGGCCACTTCTCAGCACGGGAAGTGGATGGGGACCGTTCTAA
-----+-----+-----+-----+-----+-----+ 1680
uGlnProLysProValValGlyHisPheSerAlaArgGluLeuAspGlyAspArgSerLy

40
1681 GCAGTTGGCCCTAAAGGTGCTGTGCGTTGCCAGCGGCAGTACTCCCTGCTCCAAGCAGC
-----+-----+-----+-----+-----+-----+ 1740
sGlnLeuAlaLeuLysValLeuCysValAlaGlnArgGlnTyrSerLeuLeuGlnAlaAl

45
1741 CAACCTCCTGACGCCCCGACATGATCACAGAGTTTGAGACCCTACAGCAGCTGGTGCAAGA
-----+-----+-----+-----+-----+-----+ 1800
aAsnLeuLeuThrProAspMetIleThrGluPheGluThrLeuGlnGlnLeuValGlnGl

50
1801 GGAAAAAATTGAGCCTGGGGCAGAGGCCTTGAGGACTTCAGGCCTGGCCAGGGGGGCACC
-----+-----+-----+-----+-----+-----+ 1860
uGluLysIleGluProGlyAlaGluAlaLeuArgThrSerGlyLeuAlaArgGlyAlaPr

55
1861 TCTGGCTCAGGAGCTGTGTTTCAGAGTCAATCCCTGTGCCGTCTCCTCTCTGCCAGAGCC
-----+-----+-----+-----+-----+-----+ 1920
oLeuAlaGlnGluLeuCysSerGluSerIleProValProSerProLeuCysProGluPr

Fig. 1C

5	1921	TCCAGGATACACTGGCCCTGTGACCCGGACTATGGCGAGGCGACTGAGTGGCCCCCTGCA -----+-----+-----+-----+-----+-----+-----+ oProGlyTyrThrGlyProValThrArgThrMetAlaArgArgLeuSerGlyProLeuHi	1980
10	1981	CACCCCTGGGAATCCCGCCTGGACCCAACTGCACCCAGCCCAGGGGTCCCGATGGCCCAT -----+-----+-----+-----+-----+-----+-----+ sThrLeuGlyIleProProGlyProAsnCysThrProAlaGlnGlySerArgTrpProMe	2040
15	2041	GGAGAAGAAGAGGAGGAGACCAAGCGCCTTGGAGGCAGACAGTCCCATGGCCTCAAAGCG -----+-----+-----+-----+-----+-----+-----+ tGluLysLysArgArgArgProSerAlaLeuGluAlaAspSerProMetAlaSerLysAr	2100
20	2101	GGGCACCAAGCGCCAGCGCCAGTCCTTCCTGCCCTGCCTAAGGAGAGGGTCTCTGCCTGA -----+-----+-----+-----+-----+-----+-----+ gGlyThrLysArgGlnArgGlnSerPheLeuProCysLeuArgArgGlySerLeuProAs	2160
25	2161	CACCCAACCTTCACAGGGGCCAGCACCCCCAAAGGAGAAAGGGCCTCCTCCCCCTGCCA -----+-----+-----+-----+-----+-----+-----+ pThrGlnProSerGlnGlyProSerThrProLysGlyGluArgAlaSerSerProCysHi	2220
30	2221	TTCCCCTCGCGTTTGCCAGCCACAGTCATCAAAGCCGGGTGCCCTGGGCCCTTCCGC -----+-----+-----+-----+-----+-----+-----+ sSerProArgValCysProAlaThrValIleLysSerArgValProLeuGlyProSerAl	2280
35	2281	CATGCAGAACTGCTCCACCCCGCTGGCTCTGCCCACTCGAGACCTCAATGCCACCTTTGA -----+-----+-----+-----+-----+-----+-----+ aMetGlnAsnCysSerThrProLeuAlaLeuProThrArgAspLeuAsnAlaThrPheAs	2340
40	2341	TCTCTCTGAGGAGCCTCCCTCAAAGCCCAGTTTCCATGAATGCATTGGCTGGGACAAAAT -----+-----+-----+-----+-----+-----+-----+ pLeuSerGluGluProProSerLysProSerPheHisGluCysIleGlyTrpAspLysIl	2400
45	2401	ACCCAGGAGCTGAGCAGGCTGGACCAGCCCTTCATCCCCAGGGCACCTGTGCCCTGTT -----+-----+-----+-----+-----+-----+-----+ eProGlnGluLeuSerArgLeuAspGlnProPheIleProArgAlaProValProLeuPh	2460
50	2461	CACCATGAAGGGCCCCAAGCCAACATCTTCCTCCCTGGGACCTCTGCCTGCAAGAAGAA -----+-----+-----+-----+-----+-----+-----+ eThrMetLysGlyProLysProThrSerSerLeuProGlyThrSerAlaCysLysLysLy	2520
55	2521	GCGCGTTGCGAGTTCCTCAGTCTCCCATGGCCGCAGCCGCATCGCCCGCTCCCCAGCAG -----+-----+-----+-----+-----+-----+-----+ sArgValAlaSerSerSerValSerHisGlyArgSerArgIleAlaArgLeuProSerSe	2580

Fig. 1D

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Fig. 1E

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DNA sequence of HsKIP3A, motor only

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1 GACAGCACGC TGCAAGTAGT GGTACGGGTG CGGCCCCCA CCCCTCGGGA GCTGGACAGT
61 CAGCGGCGGC CAGTGGTTCA GGTGGTGGAC GAGCGGGTGC TGGTGTTTAA CCCTGAGGAG
121 CCCGATGGAG GGTCCCTGG CCTGAAATGG GGTGGCACCC ATGATGGCCC CAAGAAGAAG
181 GGCAAAGACC TGACGTTTGT CTTTGACCGG GTCTTTGGCG AGGCGGCCAC CCAACAGGAC
241 GTGTTCCAGC ACACCACGCA CAGCGTCCTG GACAGCTTCC TCCAGGGCTA CAACTGCTCA
301 GTGTTTGCCT ACGGGGCCAC CGGGGCTGGG AAGACACACA CCATGCTGGG AAGGGAGGGG
361 GACCCCGGCA TCATGTACCT GACCACCGTG GAACTGTACA GGCGCCTGGA GGCCCGCCAG
421 CAGGAGAAGC ACTTCGAGGT GCTCATCAGC TACCAGGAGG TGTATAATGA ACAGATCCAT
481 GACCTCCTGG AGCCCAAGGG GCCCCCTGCC ATCCGCGAGG ACCCCGACAA GGGGGTGGTG
541 GTGCAAGGAC TTTCTTTCCA CCAGCCAGCC TCAGCCGAGC AGCTGCTGGA GATACTGACC
601 AGGGGGAACC GTAACCGCAC GCAGCACCCC ACTGATGCCA ACGCGACTTC CTCCCGCTCC
661 CATGCCATCT TCCAGATCTT TGTGAAGCAG CAGGACCGGG TTCCAGGACT GACCCAGGCT
721 GTCCAGGTGG CCAAGATGAG CCTGATTGAC CTGGCTGGCT CAGAGCGGGC ATCCAGCACC
781 CATGCGAAGG GGGAGCGGCT GCGGGAGGGG GCCAACATCA ACCGCTCTCT GCTGGCGCTC
841 ATCAACGTCC TCAATGCCTT GGCCGATGCA AAGGGCCGCA AGACCCATGT GCCCTACCGG
901 GACAGCAAAC TGACCCGCCT GCTCAAAGAC TCCCTCGGGG GCAACTGCCG CACAGTGATG
961 ATCGCTGCCA TCAGCCCCTC CAGCCTGACC TACGAGGACA CGTACAACAC CCTC

Fig. 2

Protein sequence of HsKIP3A, motor only

5 1 DSTLQVVVRV RPPTPRELDS QRRPVVQVVD ERVLVFNPEE PDGGFPGLKW GGTHDGPKKK
 61 GKDLTFVFDR VFGEAATQOD VFQHTTHSVL DSFLQGYNCS VFAYGATGAG KTHTMLGREG
 121 DPGIMYLTTV ELYRRLEARQ QEKHFVVLIS YQEVYNEQIH DLLEPKGPLA IREDPDKGVV
 181 VQGLSFHOPA SAEQLLEILT RGNRRRTQHP TDANATSSRS HAIFQIFVKQ QDRVPGLTQA
 241 VQVAKMSLID LAGSERASST HAKGERLREG ANINRSLAL INVLNALADA KGRKTHVPYR
 301 DSKLTRLLKD SLGGNCRTVM IAAISPSSTLT YEDTYNTL

10 Fig. 3

Figure 4:

MAVEDSTLQVVVRVRPPTPRELDSQRRPVVQVVDERVLVFNPEEPDGGFPGLKWGGT
HDGPKKKGKDLTFVFDRVFGAATQQDVQHTTHSVLDSFLQGYNCVVFAYGATGAG
KTHTMLGREGDPGIMYLTTVELYRRLEARQQEKHFEVLISYQEVYNEQIHDLLLEPKG
5 PLAIREDPDKGVVVQGLSFHQPASAEQLLEILTRGNRNRTQHPTDANATSSRSHAI
QIFVKQQDRVPGLTQAVQVAKMSLIDLAGSERASSTHAKGERLREGANINRSLLALI
NVLNALADAKGRKTHVPYRDSKLTRLLKDSLGGNCRTVMIAAISPSSTLYEDTYNTL
KYADRAKEIRLKGNSKLEGKPIPNPLLGLDSTRTGHHHHHH

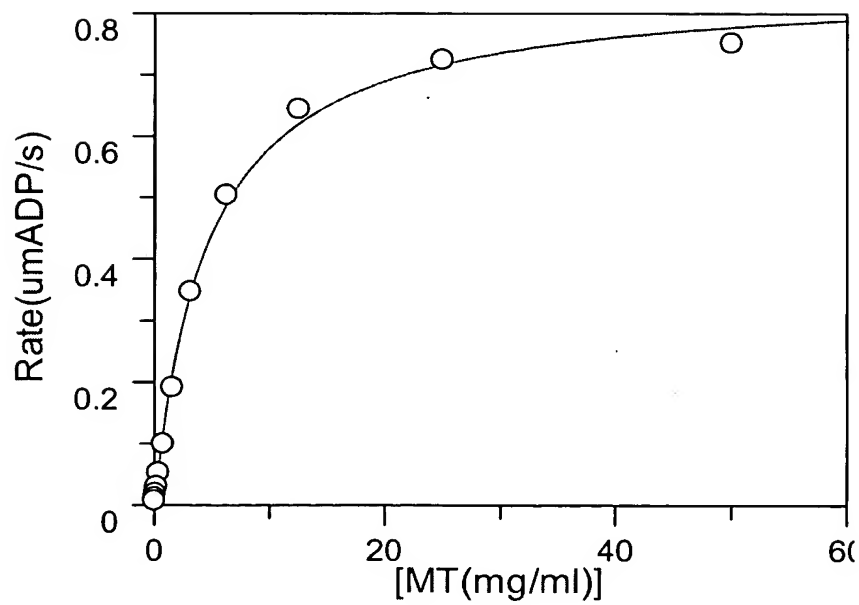
- 10 Grayed sequence is derived from the vector (pCRT7/CT, Invitrogen) and contains a V5 epitope and polyhistidine tag.

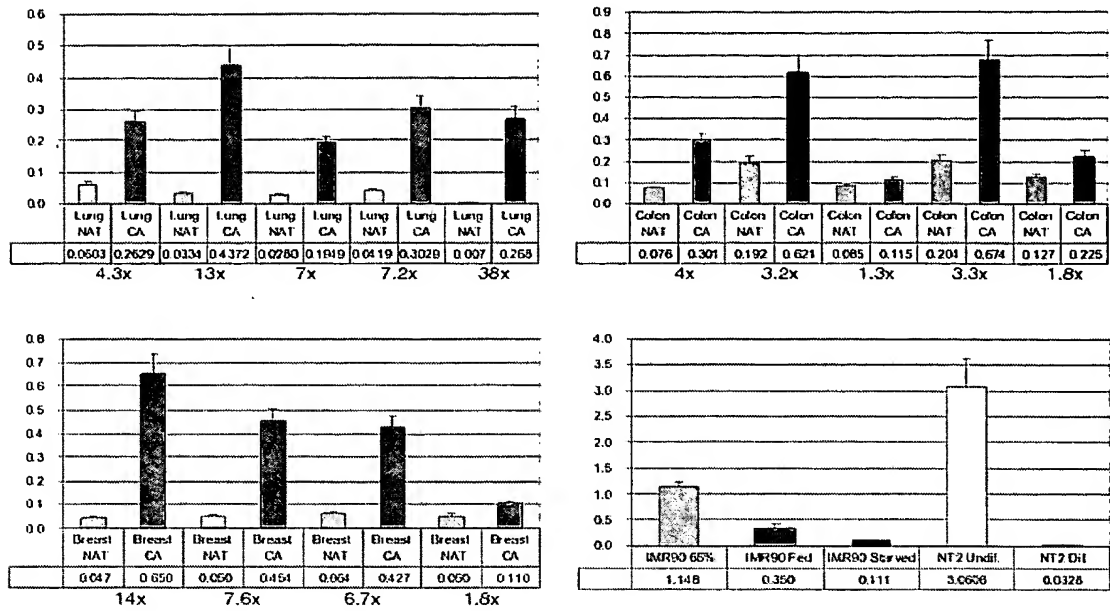
Figure 5: Nucleotide sequence of the Kip3a fragment used in the ATPase assay

ATGGCAGTGGAGGACAGCACGCTGCAAGTAGTGGTACGGGTGCGGCCCCCACCCT
CGGGAGCTGGACAGTCAGCGGCGGCCAGTGGTTTCAGGTGGTGGACGAGCGGGTGCTG
5 **GTGTTTAACCCTGAGGAGCCCGATGGAGGGTTCCCTGGCCTGAAATGGGGTGGCACC**
CATGATGGCCCCAAGAAGAAGGGCAAAGACCTGACGTTTGTCTTTGACCGGGTCTTT
GGCGAGGCGGCCACCCAAACAGGACGTGTTCCAGCACACCACGCACAGCGTCCTGGAC
AGCTTCCTCCAGGGCTACAACTGCTCAGTGTTCCTACGGGGCCACCGGGGCTGGG
AAGACACACACCATGCTGGGAAGGGAGGGGGACCCCGGCATCATGTACCTGACCACC
10 **GTGGAAGTGTACAGGCGCCTGGAGGCCCGCCAGCAGGAGAAGCACTTCGAGGTGCTC**
ATCAGCTACCAGGAGGTGTATAATGAACAGATCCATGACCTCCTGGAGCCCAAGGGG
CCCCTTGCCATCCGCGAGGACCCCGACAAGGGGGTGGTGGTGCAAGGACTTTCTTTC
CACCAGCCAGCCTCAGCCGAGCAGCTGCTGGAGATACTGACCAGGGGGAACCGTAAC
CGCACGCAGCACCCCACTGATGCCAACGCGACTTCCTCCCGCTCCCATGCCATCTTC
15 **CAGATCTTTGTGAAGCAGCAGGACCGGGTTCCAGGACTGACCCAGGCTGTCCAGGTG**
GCCAAGATGAGCCTGATTGACCTGGCTGGCTCAGAGCGGGCATCCAGCACCCATGCG
AAGGGGGAGCGGCTGCGGGAGGGGGCCAACATCAACCGCTCTCTGCTGGCGCTCATC
AACGTCCTCAATGCCTTGGCCGATGCAAAGGGCCGCAAGACCCATGTGCCCTACCGG
GACAGCAAAGTACCCGCCTGCTCAAAGACTCCCTCGGGGGCAACTGCCGCACAGTG
20 **ATGATCGCTGCCATCAGCCCCTCCAGCCTGACCTACGAGGACACGTACAACACCCTC**
AAATATGCCGACCGGGCCAAGGAGATCAGGCTCAAGGGCAATTCGAAGCTTGAAGGT
AAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGCGTACCGGTCATCATCAC
CATCACCATTGA

25 Grayed sequence is derived from the vector (pCRT7/CT, Invitrogen) and contains a V5 epitope and polyhistidine tag.

Fig. 6





5 Fig. 7

10 60086857 v1